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Plant defense against aphids, the pest extraordinaire

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Abbreviations: ACE, angiostensin-converting enzyme; *ADF3*, *ACTIN-DEPOLYMERIZING FACTOR 3*; *AFB2*, *AUXIN SIGNALING F-BOX 2*; AKR, (*Acyrthosiphon kondoi resistance*); *APR* (*Acyrthosiphon pisum resistance*); *ARF6*, A*UXIN RESPONSE FACTOR 6*; AtSEOR1, *ARABIDOPSIS THALIANA SIEVE ELEMENT OCCLUSION-RELATED 1*; *BAK1*, *BRASSINOSTERIOD INSENSITIVE 1-ASSOCIATED RECEPTOR KINASE 1*; *BIK1*, *BOTRYTIS-INDUCED KINASE 1*; DIMBOA, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one; EF-Tu, Elongation factor-thermo unstable; ETI, effector-triggered immunity; GPA, green peach aphid; GroEL, heat shock chaperone protein; HSP, heat shock protein; miRNA, microRNA; MIF (macrophage migration-inhibition factor); Mp1/PIntO1; Myzus persicae 1/Progeny Increase to Overexpression 1; PIntO2/Mp2, Myzus persicae 2/Progeny Increase to Overexpression 2; P-protein, phloem-protein; *PAD4*, *PHYTOALEXIN DEFICIENT 4*; PRR, pattern recognition receptor; PTI, pattern-triggered immunity; SEO, sieve element occlusion; SERK1, somatic embryogenesis receptor-like kinase 1; *SLI1*, *SIEVE ELEMENT-LINING CHAPERONE 1*; SGT1, Suppressor of G-two allele of Skp1; *TIR1*, *TRANSPORT INHIBITOR RESPONSE 1*; *TPS11*, *TREHALOSE-6-PHOSPHATE SYNTHASE 11*; *Vat* (*Virus Aphid Transmission*); VPS52 (Vacuolar Protein Sorting Associated Protein52)

ABSTRACT

Aphids are amongst the most damaging pests of plants that use their stylets to penetrate the plant tissue to consume large amounts of phloem sap and thus deprive the plant of photoassimilates. In addition, some aphids vector important viral diseases of plants. Plant defenses targeting aphids are broadly classified as antibiosis, which interferes with aphid growth, survival and fecundity, and antixenosis, which influences aphid behavior, including plant choice and feeding from the sieve elements. Here we review the multitude of steps in the infestation process where these defenses can be exerted and highlight the progress made on identifying molecular factors and mechanisms that contribute to host defense, including plant resistance genes and signaling components, as well as aphid-derived effectors that elicit or attenuate host defenses. Also discussed is the impact of aphid-vectored plant viruses on plant-aphid interaction and the concept of tolerance, which allows plant to withstand or recover from damage resulting from the infestation.

1. Introduction

Aphids encompass a large group of insects in the superfamily Aphidoidea (order Hemiptera) that have specialized to feed from the phloem [1]. Of the more than 4000 known aphid species, around 250 are among the most destructive pests of cultivated plants [1-3]. Aphids are r-strategists that reproduce parthenogenically. This asexual mode of reproduction, combined with a short generation time, allows aphids to rapidly attain high population densities. Further, as host quality declines, aphids produce winged morphs to facilitate dispersion to new plants. These factors along with their ability to colonize virtually any part of the plant, consume profuse amounts of photoassimilates, alter source-sink patterns, vector more than 200 viral diseases, and resistance to large number of insecticides, is what makes them pest extraordinaire [1, 4-8].

Aphids utilize olfactory and/or visual cues to find their hosts [8-12]. Once on a prospective host, aphids utilize tactile and gustatory cues, which include monitoring surface features and short probes to sample host cell contents, to ascertain suitability of the host [8, 13]. Some aphids like the cabbage aphid (*Brevicoryne brassicae*), the mustard aphid (*Lipaphis erysimi*) and the pea aphid (*Acyrthosiphon pisum*) are specialists with a limited host range covering closely related species. In contrast, the green peach aphid (GPA; *Myzus persicae*) and potato aphid (*Macrosiphum euphorbiae*) are generalists with a broad host range covering plants in different families [1, 14]. Nutritional cues likely facilitate host selection by the generalists, while plant secondary metabolites are suggested to provide cues for host selection by specialists [8, 15].

Aphid mouthparts are modified into slender stylets, which are used to pierce plant tissue and feed from the sieve elements. As depicted in Figure 1, the stylets take a largely intercellular path to the sieve elements and in the process release a gelling saliva, which provides lubrication and hardens to form a sheath [16, 17]. The sheath provides a path that facilitates smooth movement of the stylet through the intercellular spaces and simultaneously minimizes arousal of wounding responses by the host. The importance of the sheath is evident from the fact that silencing expression of the pea aphid structural sheath protein-encoding gene, *SHP*, resulted in

abnormal sheath formation that was accompanied by reduction in aphid growth and fecundity [18, 19]. On their way to the sieve elements, the stylets transiently sample the contents of epidermal and mesophyll cells to obtain cues that facilitate decisions about plant choice [20, 21]. A second type of saliva, the watery saliva, which is released when the stylet tip is inside a cell, contains proteins and metabolites that modulate host physiology to benefit the insect and facilitate sustained feeding from the sieve elements [16, 22-24]. As discussed later, some of these salivary components also elicit host defenses.

Phloem sap is sugar-rich, but contains low concentrations of essential amino acids. To cope with this nutritional imbalance in their diet, aphids have evolved diverse behavioral, anatomical and physiological adaptations. For example, aphids jettison the vast excess of sugar they consume in the 'honeydew' they excrete, and occasionally 'drink' from the xylem to aid in osmoregulation [25-30]. Furthermore, the deficit of essential amino acids in their diet is compensated by symbiosis with the γ-proteobacterium *Buchnera aphidicola* [31]. However, as discussed later, a factor derived from the symbiont also elicits plant defense to betray the aphid.

Despite the stealth nature of aphids, plants have evolved multiple strategies to resist aphids. These defenses include preformed as well as inducible factors/mechanisms. Plant defenses can be further modulated by the endosymbionts and the viruses vectored by an aphid. This review will highlight these measures and recent progress made on understanding the molecular and physiological basis of these defenses.

2. Plant defense against aphids: a conglomerate of strategies

Plant defenses against aphids, which can be categorized as antixenosis and antibiosis, are exerted at multiple levels of their interaction with aphids (Figure 1 and Table 1) [32, 33]. Antixenotic mechanisms influence aphid behavior, for example decisions about plant choice and feeding behavior. In comparison, antibiotic factors influence aphid physiology to impede growth, development, reproduction and/or survival. Strong antibiosis, however, can also affect aphid behavior.

2.1. Defenses at the plant surface

The plant surface provides the first barrier to aphids. The cell surface harbors secondary metabolites that are detrimental to insects and releases volatiles that can repel insects [34-37]. Surface waxes also influence plant-aphid interaction. For example, in oats, the presence of surface wax is critical for controlling grain aphid (*Sitobion avenae*) infestation [38, 39]. Extracts of surface wax when applied to wax-less variety of triticale deterred grain aphid settling and increased aphid mortality. Similarly, the presence of glossy leaf wax in *Brasicca oleracea* correlated with resistance to the cabbage aphid [40, 41]. Trichomes present on the plant surface also influence infestation by providing a physical barrier to insect movement [42]. In addition, glandular trichomes are a source of sugar esters and secondary metabolites like 2-tridecanone

that are detrimental to herbivores [37, 43]. Glandular trichomes also release (E)–β-farnesene, the aphid alarm pheromone, which promotes 'dispersal behavior' in aphids [44].

2.2. Defense activities in the intercellular spaces

The aphid stylets take a largely intercellular path to the sieve elements (Figure 1). In these intercellular spaces, the stylets and stylet sheath are in close contact with the plant cell surface, thus providing a site where plant defenses can target the aphid. A recent study in Arabidopsis (*Arabidopsis thaliana*) indicated that the genotype of the plant determines the amount of time it takes after tissue penetration for the stylet to find a sieve element [45]. On plants deficient in *ACTIN-DEPOLYMERIZING FACTOR3* (*ADF3*) function, the GPA stylet took less time to find sieve elements than on the wild-type plant, thus suggesting that *adf3* is deficient in a factor that controls stylet movement through the intercellular spaces. Aphid performance is also affected by changes in the redox status of the apoplast [46]. Aphid fecundity was lower on lines in which expression of an apoplastic ascorbate oxidase, which metabolizes ascorbic acid, was reduced and comparatively higher on plants overexpressing the ascorbate oxidase. Indeed, GPA infestation of tobacco results in reduction of ascorbate oxidase activity [46], thus suggesting that an overall reduction in the oxidative status of the apoplast is detrimental to the aphid.

2.3. Sieve element occlusion limits phloem sap availability to aphids

Phloem sap, which flows through the sieve elements from the source to the sink organs, is an important source of carbohydrates, amino acids, inorganic ions, secondary metabolites and hormones for the developing sink organs [47]. Aphids spend a significant portion of their time feeding from the sieve elements, thereby removing large volumes of phloem sap, thus depriving the plant tissue of important resources. Plants counter by activating processes that lead to sieve element occlusion (SEO) to impede flow of phloem contents to the aphid (Figure 1). SEO involves two processes, a relatively rapid development of proteinaceous plugs that transiently seal sieve plates followed by a slower deposition of callose that provides long-term occlusion of sieve tubes [48].

2.3.1. Contribution of phloem proteins to sieve element occlusion in response to aphid infestation

In Fabaceae, forisomes, which encompass a group of phloem (P) proteins, are involved in the occlusion of sieve elements [49]. In undisturbed sieve elements, forisomes are present as compact crystalloid spindles. In response to damage to the sieve elements, an influx of Ca^{2+} causes the volume of forisomes to increase and disperse into a spherical confirmation that seals of sieve tubes [49] [~~76~~]. In a series of elegant experiments using the electrical penetration graph technique in conjunction with confocal laser-scanning microscopy, Medina-Ortega and Walker [50] showed that feeding by the generalists GPA and potato aphid triggers forisome occlusion in faba bean (*Vicia faba*), which results in the aphids withdrawing their stylets without phloem

ingestion. In contrast, a legume-specialist, the pea aphid fails to trigger forisome occlusion [51, 52]. In experiments with the vetch aphid (*Megoura viciae*) feeding on faba bean, forisome aggregation-conferred SEO was accompanied by alteration in aphid feeding behavior, which was manifested as a switch from phloem sap ingestion to secretion of watery saliva, presumably to reverse SEO [24]. Indeed, saliva collected from the vetch aphid caused forisomes to reverse from the dispersed to the compact state, thus leading to the suggestion that salivary components have the ability to reverse and/or suppress SEO and thus facilitate feeding [24].

In contrast to the Fabaceae, contrasting reports exist for the role of P proteins in controlling aphid infestation in Arabidopsis, a Brassicaceae family plant. Arabidopsis contains two P protein-encoding genes At*SEOR1* (*ARABIDOPSIS THALIANA SIEVE ELEMENT OCCLUSION-RELATED 1*) and At*SEOR2*, which function non-redundantly in P filament formation [53]. Exudation assays in P protein-depleted Arabidopsis mutants revealed a greater loss of photosynthates [54]. However, microscopic visualization of AtSEOR1 expressed as a fusion to the yellow fluorescent protein revealed that the resulting agglomeration of AtSEOR1 was not sufficient to control flow through the phloem [55]. Further, simultaneous disruption of At*SEOR1* and At*SEOR2* did not compromise basal resistance against the GPA [53], thus suggesting that these P proteins are not critical for controlling aphid infestation on Arabidopsis. However, it is plausible that GPA may have evolved strategies to suppress the function of these SEO proteins, thus marginalizing the function of SEO proteins in limiting infestation in Arabidopsis.

A recent study that utilized genome-wide association mapping involving 350 Arabidopsis accessions for their impact on GPA feeding behavior identified a sieve element-localized small heat shock-like protein SIEVE ELEMENT-LINING CHAPERONE 1 (SLI1) as required for controlling GPA feeding [56]. Aphids fed longer, ingested phloem at a higher rate, and produced more progeny on the *sli1* mutant compared to the wild-type plant. *SLI1* does not adversely impact phloem sap exudation in non-infested plants [56]. However, whether it influences SEO in response to aphid infestation has not been tested.

2.3.2. Contribution of callose to defense against aphids

Callose is a β-1,3-glucan synthesized by callose synthases located on the plasma membrane [57]. The deposition of callose leading to the plugging of sieve plates contributes to plant defense against aphids. Similar to SEO by forisomes, an influx of Ca^{2+} into the sieve element induces the deposition of callose at sieve plates [58, 59]. Experiments with aphid saliva and whole body extracts indicate that callose deposition is stimulated by aphid-derived factors [60-63]. The Russian wheat aphid (*Diuraphis noxia*) feeding on wheat (*Triticum aestivum*) results in a massive localized deposition of callose at the feeding site leading to blockage of sieve plate pores and other symplastic connections between sieve tube members [64]. In a population of 26 diverse maize (*Zea mays*) inbred lines, it was found that lines that inherently formed less callose were more susceptible to the maize leaf aphid (*Rhopalosiphum maidis*) [65]. On melon (*Cucumis melo*) plants containing the *Vat*$^{+}$ (*Virus Aphid Transmission*) allele, which confers resistance to the cotton-melon aphid (*Aphis gossypii*), callose deposition occurred as early as 20 minutes after infestation as compared to plants lacking the *Vat*$^{+}$ allele in which callose deposition

was only observed after 72 h post infestation [66]. In Arabidopsis, infestation by the cabbage aphid induces callose deposition as early 6 h post infestation, which is sustained for up to 48 h [67]. Similarly, callose is also deposited in response to GPA infestation in Arabidopsis [45, 62, 68]. In Arabidopsis, *ADF3* function is required for controlling GPA feeding. The reduced ability of the *adf3* mutant to control aphid infestation correlated with the poor deposition of callose and the corresponding facilitation of the ability of the aphid stylets to find and to feed from the sieve elements [45], thus suggesting that an *ADF3*-dependent mechanism(s) functions at multiple stages in the infestation process to interfere with aphid infestation.

2.4. Secondary metabolites active against aphids

Plants synthesize a variety of secondary metabolites that are detrimental to aphids [69-74]. Aphids encounter these chemicals at multiple stages of the infestation process, including at the plant surface, during the short period when they sample mesophyll cell contents, and in the phloem sap they consume. Cardenolides belong to a family of steroidal cardiac glycosides that are present in the phloem of a wide variety of plant species [69, 70]. They specifically inhibit Na^+/K^+ ATPases. N-containing cyclic alkaloids, which are found in 20-30% of all plants, adversely impact cellular processes like DNA replication, protein synthesis and neurotransmission [69, 71, 72]. The S-containing glucosinolates are major defensive metabolites in Brassicaceae, which when acted upon by myrosinase release products that are rapidly converted to toxic thiocyanates and/or nitriles [73]. Glucosinolates and myrosinases are sequestered into different plant cells, thus requiring damage of the two cell types to bring the myrosinases in contact with the glucosinolates. However, due to the minimal physical damage to plant tissues caused by stylets, aphids consume and excrete the glucosinolates largely intact, thus avoiding their toxic effects [74]. Among the indole and aliphatic glucosinolates, the indole glucosinolates, which are less stable and spontaneously converted to toxic metabolites are most effective against aphids like the generalist GPA [74, 75]. In comparison, specialists like the cabbage aphid have evolved strategies to sequester glucosinolates and thus bypass their toxic effects [76]. 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one glucoside (DIMBOA-Glc) is the most abundant benzoxazionid in maize seedlings. When acted upon by glucosidases it releases insect-deterrent compounds like 6-methoxybenzoxalin-3-one [65, 69]. DIMBOA as well as 4-methoxyindole-3-ylmethylglucosinolate also stimulate deposition of callose [77, 78], which as described above, contributes to sieve element occlusion.

2.5. Antimetabolic lectins and protease inhibitors

Phloem sap contains lectins, which specifically bind to carbohydrates in the insect gut and interfere with physiological processes to adversely impact insect health [79-82]. Indeed, the lectin Phloem Protein2-A1 (PP2-A1) from Arabidopsis, when included in a synthetic diet, adversely impacted weight gain by GPA and soybean aphid (*Aphis glycines*) [83]. Constitutive expression of lectins from a variety of plant sources also has a beneficial effect on plant resistance to aphids [84-87]. Non-protein amino acids, for example *L*-DOPA (*L*-3,4-dihydroxyphenylalanine) and N^{δ}-acetylornithine also function to deter aphid infestation [88, 89].

Plants contain a variety of protease inhibitors, which function to control activity of endogenous proteases [90]. Some of these protease inhibitors also contribute to defense against herbivores by inhibiting activity of gut proteases required for digesting ingested proteins. Considering their diet, in theory aphids should not require proteolytic digestion in the gut for their nutritional requirement. However, proteases are present in the gut lining of aphids, where they are likely involved in processing ingested polypeptides [80]. Protease inhibitors could target these proteases. Indeed, population growth and fecundity of the pea aphid, cotton-melon aphid, potato aphid and GPA were lower when fed on a diet containing cystatin family of protease inhibitors [91, 92]. However, the targets of these protease inhibitors in aphids are unknown. Aphidicidal activity is also exhibited by a Bowman Birk-type protease inhibitor derived from the pea plant [93, 94]. However, there is no evidence that serine-proteases, which are targets of Bowman Birk-type protease inhibitors, are present in aphid gut. Hence, whether these protease inhibitors act in the insect gut or elsewhere in the aphid remains to be determined.

3. Perception of aphids

Since the classical study by Harold Flor [95] resulting in the "gene-for-gene" model for plant resistance to pathogens and the subsequent evolution of the multilayered "zig-zag" model for plant immunity against pathogens [96], similar models have been applied to the study of plant-herbivore interaction [22, 23, 97, 98]. Based on these models, immune receptors in the plant perceive pest-derived factors, resulting in the subsequent elicitation of immune responses. Plants have evolved pattern recognition receptors (PRRs) to recognize molecular patterns conserved across a larger group of microbes. These pathogen/microbe-associated molecular patterns (PAMPs/MAMPs), for example the bacterial flagellar protein-derived flg22 peptide, upon recognition by the cognate PRR, result in pattern-triggered immunity (PTI) contributing to non-host resistance. However, some pathogen races express effectors that suppress the sustained activation of PTI to facilitate infection. Plants have further evolved Resistance (R) proteins to recognize these race-specific effectors to elicit a more robust effector-triggered immunity (ETI). As illustrated in Figure 2, the emerging paradigm for plant immunity to insects suggests that plants recognize conserved herbivore-associated molecular patterns (HAMPs) to activate defenses. Further, insects release effectors that suppress activation of HAMP-triggered defenses, and plants have evolved R proteins that specifically recognize these effectors to turn on ETI [22, 23, 97, 98].

3.1. Resistance genes in plant defense against aphids

Loci conferring aphid biotype-specific resistance have been described in tomato (*Solanum esculentum*), melon, barrel medic (*Medicago truncatula*), and soybean (*Glycine max*). However, only two of these genes have been cloned. The *Mi-1.2* gene in tomato and the *Vat* gene in melon confer resistance against the potato aphid and the cotton-melon aphid, respectively [99-101]. Both genes encode coiled-coil (CC)-nucleotide-binding site (NBS)-leucine-rich repeat (LRR) type R proteins. In addition, *Mi-1.2* confers resistance to other insects such as whiteflies and psyllids, which have similar feeding strategy as of aphids, and certain species of root-knot

nematodes [23]. The *Vat* locus adversely influences feeding by the cotton-melon aphid and confers resistance to the viruses transmitted by cotton-melon aphid [102, 103]. However, the resistance to viruses transmitted by cotton-melon aphid is aphid-clone dependent [102]. There are suggestions that the *Vat* locus contains at least two linked genes, *Vat-1* and *Vat-2*, with *Vat-1* being the cloned *Vat* gene [103]. Whereas Mi-1.2 was localized to plasma membrane, cytoplasm and nucleus [104], Vat is predicted to be located only in the cytoplasm [101].

In *M. truncatula*, resistance to the Australian pea aphid biotype is a dominant trait conferred by the *Acyrthosiphon pisum resistance* (*APR*) locus, while resistance to the closely related bluegreen aphid *A. kondoi* is conferred by the *AKR* (*Acyrthosiphon kondoi resistance*) locus [105, 106]. These genes are located in a region that is rich in nucleotide binding site NBS-LRR type *R* genes. Biotype-specific resistance against soybean aphid conferred by *Rag* (*Resistance against Aphis glycines*) has been mapped to four chromosomes in soybean [107-113]. However, the identity of the *APR*, *AKR* and *Rag* genes, and the corresponding elicitor, remains to be determined.

3.2. Elicitors of plant defense

Chitin, a polymer composed of β-(1,4)-linked *N*-acetyl-D glucosamine (GlcNac) is a fungal MAMP recognized by lysin motif (LysM)-containing receptors in plants [114]. Chitin is a component of the aphid exoskeleton and stylet [115]. However, whether chitin is an aphid MAMP is unclear. Furthermore, whether any of the known chitin receptors are involved in plant defense against aphids is not known.

Saliva is one of the first aphid secretion that infested plant tissues encounter. The watery saliva secreted by aphids contains several proteins, some of which have well defined biochemical activities, including pectinases, cellulases, polyphenoloxidases, peroxidases, and lipases that likely facilitate infestation [116]. GPA saliva when applied to plant tissue limited GPA population, thus indicating that it is a source of plant defense elicitors, as well [117]. This active factor was determined to be proteinaceous and contained within a 3- to 10-kDa fraction. The availability of aphid genome sequence and proteomics tools has facilitated the identification of salivary proteins [60, 118-128]. Transient expression approaches combined with studies in transgenic plants have facilitated testing the elicitor function of some of these aphid salivary proteins (Table 2). For example, the genes encoding the GPA salivary proteins Mp10, Mp42, Mp56, Mp57, and Mp58 when expressed in plants, resulted in significant reduction in GPA fecundity [61, 118]. However, the biochemical function of these proteins is unknown. Expression of the potato aphid salivary protein Me47 in Arabidopsis also significantly reduced GPA fecundity [129]. Recombinant Me47 possesses glutathione *S*-transferase activity [129]. However, the relationship between its glutathione *S*-transferase activity and its ability to elicit defense against GPA in *N. benthamiana* is unclear.

The potato aphid salivary secretome contains the endosymbiont *Buchnera*-derived chaperonin GroEL [60]. GroEL effectively triggered plant defense mechanisms, including oxidative burst, callose deposition, and the expression of PTI marker genes [60]. Furthermore, aphid performance was reduced on *N. tabacum*, tomato and Arabidopsis plants that constitutively expressed GroEL [60, 61]. *BAK1* (*BRASSINOSTERIOD INSENSITIVE 1-ASSOCIATED*

RECEPTOR KINASE 1), which is an important co-receptor in PTI that targets microbial diseases, is also required for GroEL-induced oxidative burst and callose deposition, thus suggesting that some molecular components are shared between plant defenses elicited by pathogens and aphid-associated microbes. Microbes are also present in honeydew and several bacterial proteins, including EF-Tu (Elongation factor-thermo unstable) and flagellin, are detected in aphid honeydew [130], thus suggesting that microbes present in the honeydew also could potentially influence plant-aphid interaction.

3.3. Aphid salivary proteins facilitate infestation and suppress plant defense

Aphid saliva contains proteins that facilitate infestation (Table 3). The salivary protein, C002, was first described for pea aphid [131]. Although the molecular function of C002 is unknown, C002 is a critical salivary protein required for sustained feeding by the aphids. Knockdown of C002 in pea aphid adversely impacted feeding and colonization [132]. Orthologs of C002 are present in other aphid species, including the cotton-melon aphid, GPA, brown citrus aphid (*Toxoptera citricida*), and greenbug (*Schizaphis graminum*) [133]. C002 orthologs in different aphid species exhibit sequence variability and are highly species-specific. For example, while overexpression of the GPA C002 in *Nicotiana benthamiana* or in Arabidopsis resulted in enhanced GPA fecundity [118, 134], overexpression of the pea aphid C002 failed to enhance GPA fecundity in Arabidopsis [134].

In vertebrates, macrophage migration-inhibition factors (MIFs) are cytokines that modulate innate immunity and inflammation. The pea aphid genomes contain five genes that are homologous to MIFs and are likely involved in insect immunity [62]. RNAi-mediated knockdown of Ap*MIF1*, which is normally expressed in salivary glands, adversely impacted the survival, fecundity and the ability of pea aphid to feed from faba bean plants. Similarly, RNAi-mediated suppression of MpMIF1, the GPA homolog, also resulted in reduced survival and fecundity of the GPA. Like Ap*MIF1*, Mp*MIF1* is expressed in the salivary glands of GPA. Transient expression of Mp*MIF1* in *N. benthamiana* restored survival and fecundity in the Mp*MIF1* RNAi aphid, thus confirming an important function of MpMIF1 delivered into the plant in facilitating colonization of plants by the aphid [62]. When transiently expressed in *N. benthamiana*, Mp*MIF1* suppressed the induction of programmed cell death, callose deposition and pathogenesis-related gene expression by cryptogein, a plant defense elicitor from the oomycete pathogen *Phytophthora cryptogea* [62], thus indicating that aphid MIFs potentially target host defense machinery for suppression (Figure 2). However, whether the MIFs similarly impact host defense machinery as part of the infestation process remains to be determined.

The Ca^{2+}-binding protein Armet was identified from the salivary glands of pea aphids [135]. Armet is delivered into the host plant by the aphid. *Armet* expression is higher in salivary glands of insects feeding on plants than on artificial diet, thus suggesting that it might have a more important function when aphids are feeding on plants. RNAi-mediated knockdown of *Armet* had an adverse impact on insect feeding and life span on faba beans, thus suggesting that *Armet* is required for controlling aphid feeding from plants, presumably due to its impact on processes in the plant. However, the application of Armet to plant tissue resulted in the activation of transcriptional responses that are normally associated with plant response to pathogen, thus

suggesting that Armet, or a product of Armet, might also have an elicitor function in plants. However, it is plausible that Armet-triggered physiological changes in plant defense is a ploy utilized by the aphid to trick the host. In mammals and insects, Armet is a bifunctional protein, which is involved in the intracellular unfolded protein response and as an extracellular neurotrophic factor. The relationship between these biochemical properties of Armet and its ability to facilitate aphid feeding from plants remains to be evaluated. Similar to Armet, proteins related to angiotensin-converting enzymes (*ACE1* and *ACE2*) have been identified in the salivary secretions of pea aphid [136]. Simultaneous knockdown of *ACE1* and *ACE2* in the insect enhanced mortality on plants. However, aphid feeding from the sieve elements was higher in the double knockdown insects. In mammals, ACEs, which hydrolyze dipeptides from the C-terminus of short peptides, are involved in processing of neuropeptides and hormones [137]. Since *ACE2* is expressed in the brain, ovary and gut of pea aphid, in addition to the salivary glands [136], the effects of doubly knocking down *ACE1* and *ACE2* could likely be due to their impact on insect physiology, *per se*. Whether ACEs are deposited into plant tissue and potentially process plant peptides to facilitate feeding, is not known.

Several salivary proteins target physiological/molecular processes in the host to promote aphid fecundity. For example, the Mp1/PIntO1 (Myzus persicae 1/Progeny Increase to Overexpression 1) and Mp2/PIntO2 (Myzus persicae 3/Progeny Increase to Overexpression 2) proteins from GPA when constitutively expressed in Arabidopsis, increased GPA performance [118, 134]. Expression of the GPA Mp1 in the phloem was sufficient to promote GPA colonization of Arabidopsis [138]. However, the pea aphid homolog of Mp1 and Mp2 were unable to promote GPA fecundity on Arabidopsis, thus suggesting pest-specific role for these insect proteins [134]. In plants, Mp1 association with Vacuolar Protein Sorting Associated Protein52 (VPS52) results in the relocation of Mp1 to vesicle like structures that associate with prevacuolar membranes. Since VPS52 levels were negatively associated with aphid virulence and VPS52 levels decline in response to GPA infestation, it has been suggested that VPS52 is a virulence target [138]. GPA fecundity also increased when the salivary proteins Mp55 and MpC002 were transiently expressed in *N. tabacum* and stably expressed in transgenic Arabidopsis [61]. In contrast, RNAi-mediated silencing of Mp55 in aphids resulted in reduced virulence on plants. Mp55 suppresses host defenses (Figure 2). The accumulation of callose, H_2O_2 and 4-methoxyindol-3-ylmethylglucosinolate was significantly lower in GPA-infested Mp55 expressing plants compared to the GPA-infested non-transgenic controls [61]. Similarly, aphid fecundity was enhanced in plants expressing the potato aphid salivary proteins Me10, Me23 and Me47 [129, 139]. Except for Me47, which encodes a glutathione-S-transferase [129], the molecular function of the other salivary proteins is unknown.

Besides saliva, honeydew also contains factors that suppress host defenses. Honeydew collected from pea aphid-infested faba bean suppressed the activation of jasmonate responses [140]. Although the factors in honeydew responsible for suppressing jasmonate responses remain to be identified, this could be a potential mechanism that contributes to the suppression of JA responses during pea aphid infestation.

4. Plant defense signaling

The plant hormones ethylene, jasmonic acid, salicylic acid and abscisic acid contribute to signaling associated with plant-aphid interaction. However, aphids modulate hormonal signaling to benefit the insect. Readers are directed to recent reviews that have discussed the contribution of hormones in plant-aphid interaction [68, 141, 142].

Although the aphid effectors perceived by *Mi-1.2, Vat, APR, AKR* and *Rag* in plants remain to be identified, significant progress has been made in understanding the signaling machinery associated with *Mi-1.2*-conferred resistance to potato aphid in tomato. Some genes that are involved in ETI to microbes are also required for *Mi-1.2*-conferred resistance to potato aphid. These include, HSP90 (Heat shock protein 90) and SGT1 (Suppressor of G-two allele of Skp1) [143]. Comparable to their role as chaperones in plant defense against microbes, HSP90 and SGT1 are suggested to ensure proper folding and/or stability of Mi-1.2. A receptor-like kinase encoded by the tomato *SERK1* (*SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE 1*), as well as a mitogen-activated protein kinase cascade and the transcription factors WRKY70 and WRKY72 are also required for *Mi-1.2*-conferred resistance [144-146].

As indicated above and illustrated in Figure 2, BAK1, which is a co-receptor in PTI, is required for the GroEL-induced resistance against aphids [60]. *BAK1* also contributes to non-host resistance to pea aphid in Arabidopsis [63]. Pea aphid longevity was longer on the *bak1-5* mutant compared to wild-type Arabidopsis. *BAK1* is required for Ca^{2+} fluxes that occur in response to GPA probing of Arabidopsis leaves [147]. Ca^{2+} is a secondary messenger in eukaryotes, including plants. It is widely believed that Ca^{2+} has a role in phloem occlusion via its effect on callose deposition and promoting phloem protein aggregation [59, 147-149]. Genes associated with Ca^{2+} signaling exhibit altered expression in aphid-infested, compared to non-infested plants [150]. In Arabidopsis-GPA interaction, these Ca^{2+} fluxes were observed in epidermal and mesophyll cells [147]. The Ca^{2+} flux was not restricted to cells around stylet probing site, but rather covered cells beyond the probing site. This influx of Ca^{2+} into the cytoplasm required the plasma membrane ion channels GLUTAMATE RECEPTOR-LIKE 3.3 and 3.6 (GLR3.3 and GLR3.6) as well as an endomembrane ion channel TWO-PORE CHANNEL1 (TPC1), thus suggesting apoplastic as well as vacuolar sources of this Ca^{2+} [147]. However, GPA feeding and fecundity was not affected in mutants deficient for these ion channels or BAK1, thus suggesting either that *BAK1* and these ion channels are not critical for controlling GPA infestation, or alternatively, the GPA might suppress defense signaling downstream of these ion channels.

In PTI, BAK1 phosphorylates BIK1 (BOTRYTIS-INDUCED KINASE 1) leading to the activation of defenses [151, 152]. However, studies in Arabidopsis indicate that BIK1 negatively controls plant defenses against GPA [67], thus suggesting differences between the involvement of BAK1 and BIK1 in plant defense against aphid compared to PTI. As illustrated in Figure 2, the repressive effect of BIK1 on Arabidopsis defense against the GPA is due to it negatively controlling expression of *PAD4* (*PHYTOALEXIN-DEFICIENT4*) [67]. *PAD4* is required for deterring insect settling on the plant, for the accumulation of an antibiosis factor in the vascular sap, for deterring insect feeding from the sieve elements, and for callose deposition [45, 153-156]. Further, *PAD4* promotes premature leaf senescence in aphid-infested leaves, which likely limits the long-term availability of nutrients and adversely impacts the quality of the tissue for the insect. *PAD4* is also required for PTI and ETI against pathogens. However, genetic studies indicate that PAD4's contribution to defense against aphids is distinct from its involvement in

defense against pathogens [154, 156]. Not only is ENHANCED DISEASE SUSCEPTIBILITY1, which is a molecular partner of PAD4 in defense against pathogen, not required for defense against the GPA, but amino acid S118 in PAD4, which is required for PAD4 function in controlling aphid infestation, has no discernible requirement in PAD4's role in defense against pathogen. It was suggested that distinct molecular activities of PAD4 are involved in defense against pathogens and aphids [154, 157].

Various pathways that impact defense activation converge on *PAD4*. As mentioned above a *BIK1*-depdendent pathway negatively regulates *PAD4* expression. In contrast, a trehalose-dependent mechanism is required for the timely upregulation of *PAD4* in response to GPA infestation (Figure 2) [158]. *PAD4* upregulation was delayed in the *tps11* mutant, which is deficient in TREHALOSE PHOSPHATE SYNTHASE 11 activity, which is required for the increase in trehalose that occurs in response to GPA infestation. Like the *pad4* mutant, the *tps11* mutant also exhibited increased susceptibility to the GPA, which in part was due to increased feeding activity of the insects on the *tps11* mutant compared to the wild-type plant. Trehalose, a non-reducing disaccharide, and its precursor trehalose-6-phosphate, are signaling metabolites in plants [159, 160]. Indeed, trehalose application induces *PAD4* expression and enhances resistance to the GPA [158].

PAD4 expression, which is under positive feedback regulation, is controlled by an *ADF3*-dependent mechanism (Figure 2) [45]. The aphid infestation-associated upregulation of *PAD4* was delayed in *adf3*, which like the *pad4* mutant, exhibits increased susceptibility to the GPA. Constitutive expression of *PAD4*, restored resistance in the *adf3* mutant, thus confirming an important role for *PAD4* in the *ADF3*-determined resistance mechanism. ADF3 is involved in actin cytoskeleton reorganization, thus suggesting a link between the ADF3-dependent actin cytoskeleton reorganization and plant defense against the GPA. Indeed, resistance in the *adf3* mutant was restored by the application of the actin cytoskeleton destabilizers cytochalasin B and latrunculin D [45], thus further suggesting a role for the actin cytoskeleton reorganization in plant defense against the GPA.

5. Role of small RNAs in plant defense against aphids

MicroRNAs (miRNAs), which are one of the major classes of non-coding small RNAs, are involved in the regulation of gene expression, transcript stability, as well as translational control. Differential accumulation of miRNA has been reported in resistant plants challenged with aphids. For example, in *Chysanthemum morifolium*, resistance to chrysanthemum aphid (*Macrosiphoniella sanbourni*) was accompanied by differential accumulation of miRNA [161]. In wheat, resistance to the Russian wheat aphid biotype 1 conferred by the *Dn1* resistance gene was also accompanied by alterations in accumulation of miRNA [162]. While studying Arabidopsis interaction with GPA, Kettles et al. [163] found that Arabidopsis mutants defective in the synthesis of small RNA showed altered resistance to GPA. In particular, the *dcl1* (*dicer-like1*) mutant, but not plants lacking *dcl2*, *dcl3* or *dcl4*, exhibited enhanced resistance to GPA. Aphid fecundity was also adversely impacted on the *ago1* (*argonaute 1*) mutant, which is deficient in an important component of the RNA-induced silencing complex. Similarly, aphid fecundity was reduced on mutants affecting function of other genes involved in miRNA processing and transport [163]. The resistance phenotype of the *dcl1* mutant was paralleled by

increased expression of genes involved in the synthesis of glucosinolates and the phytoalexins, camalexin [163].

In case of cotton-melon aphid infestation of melon, differential accumulation of miRNA was observed amongst the insect-infested *Vat*$^{+}$ (resistant) compared to *Vat*$^{-}$ (susceptible) melon cultivars [164]. Some targets of these miRNA include genes involved in regulating the synthesis and signaling by various phytohormones, including auxin. The auxin-miRNA interactome indicated that a change towards down-regulation of auxin signaling is one of the factors that contributes to *Vat*-mediated resistance to cotton-melon aphid in melon. In particular, a *Vat*-dependent miR393-mediated downregulation of the auxin receptors TIR1 (TRANSPORT INHIBITOR RESPONSE 1) and AFB2 (AUXIN SIGNALING F-BOX 2), and miR167-mediated repression of ARF6 (AUXIN RESPONSE FACTOR 6) and ARF8 (AUXIN RESPONSE FACTOR 8), which encode transcription factors in auxin signaling was predicted [165]. Indeed, expression of auxin-regulated genes was down-regulated as early as 12 h after aphid infestation in the resistant plants. Furthermore, the application of a chemical inhibitor (PEO-IAA) of the auxin receptor to leaves of the susceptible melon variety conferred a reduction in aphid fecundity, thus implicating miRNA-mediated downregulation of auxin signaling in defense against aphid in melon [165].

6. Modulation of plant-aphid interaction by aphid-associated viruses

The impact of virus infection on host response and vector behavior depends on the interaction between the virus and its vector. In general, this interaction can be categorized into two groups. In non-circulative transmission the virus is acquired within seconds attaching itself to the aphids' mouthparts [166] and is released when the aphid feeds upon a different healthy plants. In circulative transmission the ingested virus passes through the gut lining to enter the hemolymph from where it is plausibly circulated through the entire body (and sometimes replicate). Upon reaching the salivary glands, the virus is released into the saliva from where it is transmitted to plants. Both circulatively-transmitted and non-circulatively-transmitted viruses have contrasting effects on vector settling, performance and feeding preferences. Non-circulatively-transmitted viruses tend to reduce host plant quality to promote rapid dispersion of the vector, whereas circulatively-transmitted viruses benefit from improving host quality to promote long-term feeding by the vector and improve the vector's chance of acquiring the virus [167].

Plant viruses cause morphological, physiological, biochemical and molecular changes in their host plants to alter their suitability for the vector [168]. As a first step, plant viruses upon infection of the host alter the volatile organic compound blend to attract insect vectors. Studies using the potato-GPA-*Potato leaf roll virus* pathosystem have shown that viruliferous aphids i.e. *Potato leaf roll virus* carrying GPA prefer to settle on noninfected plants whereas non-viruliferous aphids tended to settle on *Potato leaf roll virus*-infected plants [169]. Similar behavior of aphid has been observed in the wheat–*R. padi*–*Barley yellow dwarf virus* pathosystem. Nonviruliferous adults of *R. padi* are attracted to *Barley yellow dwarf virus*-infected wheat in response to a volatile organic compound blend produced by the infected plant and viruliferous adults are attracted to noninfected wheat plants [170, 171]. In some plant–virus–

aphid interactions, the presence of viruses negatively affects the performance of the vector. On wheat, *Barley yellow dwarf virus* infection leads to a less efficient use of phloem sap by the grain aphid [172].

Plant-virus interaction can also influence plant defense and thus impact aphid performance. For example, in Arabidopsis and tobacco plants infected with *Turnip mosaic virus*, the fecundity of the vector GPA is higher than on non-infected controls. *Turnip mosaic virus* infection resulted in the suppression of callose deposition and led to an increase in abundance of free amino acids [173], thus suggesting that the virus alters host physiology to promote transmission. A single *Turnip mosaic virus* protein, NIa-Pro (Nuclear Inclusion a - Protease domain) is responsible for changes in host physiology [173] and for suppressing Arabidopsis defense against GPA by repressing the ethylene signaling pathway [174]. A second *Cucumber mosaic virus* protein 2b, interferes with jasmonic acid signaling which could possibly promote aphid infestation [175-177]. A similar synergistic effect on potato aphid fecundity and abundance was observed in tomato plants infected with *Potato virus Y* [178]. On the other hand, in case of *Cucumber mosaic virus* infection of squash plants, an increase in salicylic acid and concomitant reduction in jasmonic acid biosynthesis as well as sensitivity was observed, which was dependent on virus titer and detrimental to the aphid [179, 180].

7. Tolerance as a means to minimize damage resulting from aphid infestation

Unlike antibiosis and antixenosis, plant tolerance has received little attention [33, 181, 182]. Although there are developing arguments and justifications for understanding the mechanisms of plant tolerance to aphids [181], few have met the challenge. Unlike antibiosis and antixenosis that affect the biology and behavior of the insects, tolerance involves only plant characteristics [33, 181]. This category of resistance has the unique ability in which a plant can withstand or recover from damage by insect populations [33]. Furthermore, tolerance aids in decreasing apparent plant injury and thereby crop losses, while exerting less selection pressure on the insects to form new biotypes.

Identifying and characterizing tolerance mechanisms are extremely difficult, partly because tolerant plants will support higher insect populations similar to those on a susceptible cultivar and several plant physiological parameters (e.g., stomatal conductance, water balance) that potentially contribute to tolerance trait(s), which are not visually detectable, maybe overlooked during screening for tolerance. For instance, a tolerant soybean genotype exhibits tolerance phenotype to soybean aphids as early as V3 vegetative stage and upholds as plants mature [183]. However, early vegetative stage (V1) soybean plants did not exhibit tolerant phenotype to soybean aphids. Furthermore, different aphid pressures (low vs. high) introduced at the V3 stage did not impact the yield parameters of tolerant soybean genotype, whereas different aphid pressures negatively affected both V1 and V3 stages of susceptible genotype [183]. These results suggest that developmental stages of plants play a critical role in tolerance phenotype.

Although the underlying mechanisms that contribute to tolerance are mostly unknown, based on the available literature, Koch et al. [181] proposed two main mechanisms for plant tolerance, enhanced photosynthetic compensation and reactive oxygen species scavenging. For

example, aphid tolerant plants had enhanced photosynthetic activity as a compensatory mechanism and elevated levels of reactive oxygen species scavenging enzymes that can readily detoxify insect-feeding induced reactive oxygen species [184-188]. Reactive oxygen species and derived molecules can serve as signaling molecules that are involved in altering plant defenses to biotic stress [189]. However, the downstream divergence of reactive oxygen species signaling pathway that contributes to plant tolerance phenotype is not known. Similarly, far less is known about the role of phytohormones in plant tolerance to aphids.

8. Conclusion

Despite the importance of aphids as important agricultural pests, our understanding of plant defense mechanisms against aphids has lagged that of plant defense against pathogens, as well as lepidopterans. However, significant strides made in recent years have resulted in the identification of resistance genes and signaling mechanisms that contribute to defense, and aphid-derived elicitors of plant defense. In addition, aphid effectors that suppress plant defenses have been identified. A common paradigm is emerging on how plants perceive aphid and pathogen. In addition, some signaling components are shared between plant defense against aphids and pathogen. However, significant differences also exist in how these components engage downstream signaling machinery. The availability of plant and aphid genome sequences and the development of tools to silence aphid gene expression should increase the speed of discovery in this field and aid in the development of novel strategies to combat aphid infestation of plants.

Conflict of Interest

The authors declare no conflict of interest.

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Figure Legends

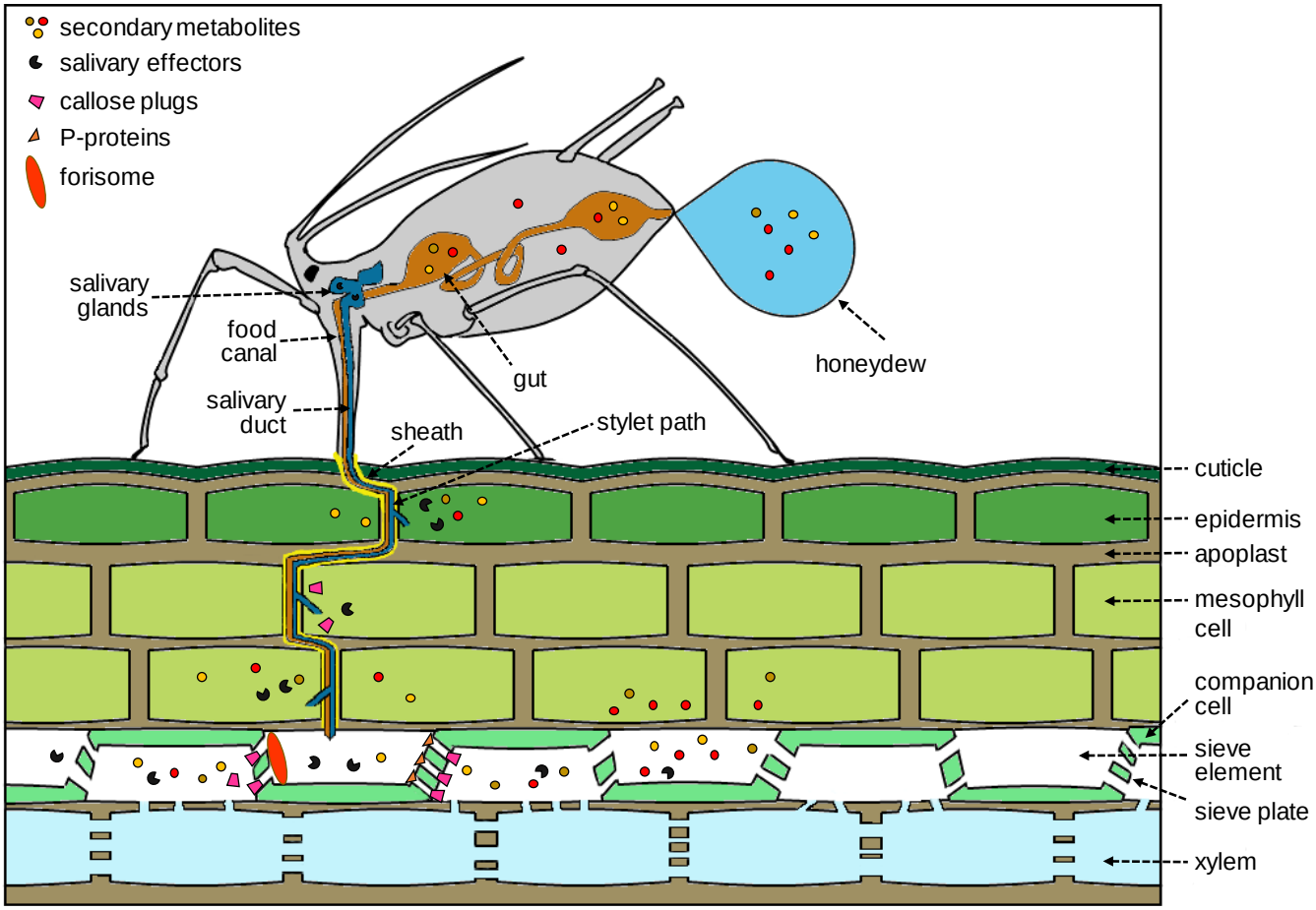
Figure 1. Illustration of an aphid feeding and the plant defenses that it encounters.

An aphid is shown with its stylet penetrating the plant tissue. The sheath formed upon hardening of the gelling saliva encases the stylet to provide a path that facilitates stylet movement. The stylets, which take a largely intercellular route to the sieve element, occasionally sample the contents of the epidermal and mesophyll cells for gustatory cues that allow it to make decisions on whether to continue feeding. When the stylet tip penetrates a cell, it releases a watery saliva, which contains effectors that facilitate feeding. Some of these effectors are recognized by the plant to elicit defenses. The plant surface components, including the waxy cuticle and trichomes (not shown), which release deterrents as well as toxic metabolites, provides the first line of defense. Once the stylet penetrates the tissue, the apoplastic space provides another site where the stylet can encounter defenses (e.g. callose and alterations in redox status). Factors that are detrimental to the insect's physiology (e.g. secondary metabolites) are acquired when the insect occasionally samples cells contents and when it feeds from the sieve elements. Sieve element occlusion resulting from changes in the physical state of the forisomes and P proteins, and the deposition of callose, can further limit access to phloem sap by the aphid. Besides, the aphid saliva, factors contained in the honeydew expelled by the aphid can also elicit or modulate host defenses.

Figure 2. Signaling associated with plant defense against aphid.

This model is based primarily on studies with the Arabidopsis-green peach aphid and the tomato-potato aphid pathosystems. The emerging paradigm for plant immunity to aphids is that plant cells utilize a plasma membrane-localized pattern recognition receptor (PRR) to perceive a conserved herbivore-associated molecular pattern (HAMP) to activate pattern-triggered immunity (PTI). Aphid whole body extracts as well as the microbial GroEL protein can stimulate plant defenses, including defense gene expression and callose deposition. BAK1, which functions as a co-receptor in PTI, is required for the induction of defenses by aphid extracts and GroEL. Aphid whole body extract induced defense involves an influx of Ca^{2+} from apoplastic as well as vacuolar stores. However, how this Ca^{2+} stimulus is relayed leading to the activation of defenses is not known. Aphid-derived effectors, for example, Mp55 and MIF1, are shown to attenuate a subset of defenses associated with PTI. Plants have also evolved Resistance (R) proteins that are involved in the specific recognition of pest-derived effectors that elicit effector-triggered immunity (ETI). In tomato, the R protein Mi1.2, which confers resistance against potato aphid, requires a plasma membrane-localized receptor-like kinase (RLK) SERK1. Arabidopsis PAD4 is a nucleocytoplasmic protein, which is involved in PTI and ETI against pathogens. PAD4 is also essential for basal resistance against the green peach aphid. PAD4 function is required for deterring aphid feeding, callose deposition, the activation of cell death and premature leaf senescence, and accumulation of an antibiotic factor. However, how the PAD4 arm of defense integrates with PTI against aphids is unclear. Moreover, whether PAD4 is required for ETI against aphids is not known. *PAD4* expression, which is upregulated in response to green peach aphid infestation, is regulated by a positive feedback mechanism, which requires ADF3, thus suggesting a role for actin cytoskeleton dynamics in regulation of defense

gene expression. *PAD4* expression is also positively regulated by TPS11 and its product trehalose, and is negatively controlled by BIK1, thus indicating that *PAD4* is a node where multiple defense regulatory signals converge.



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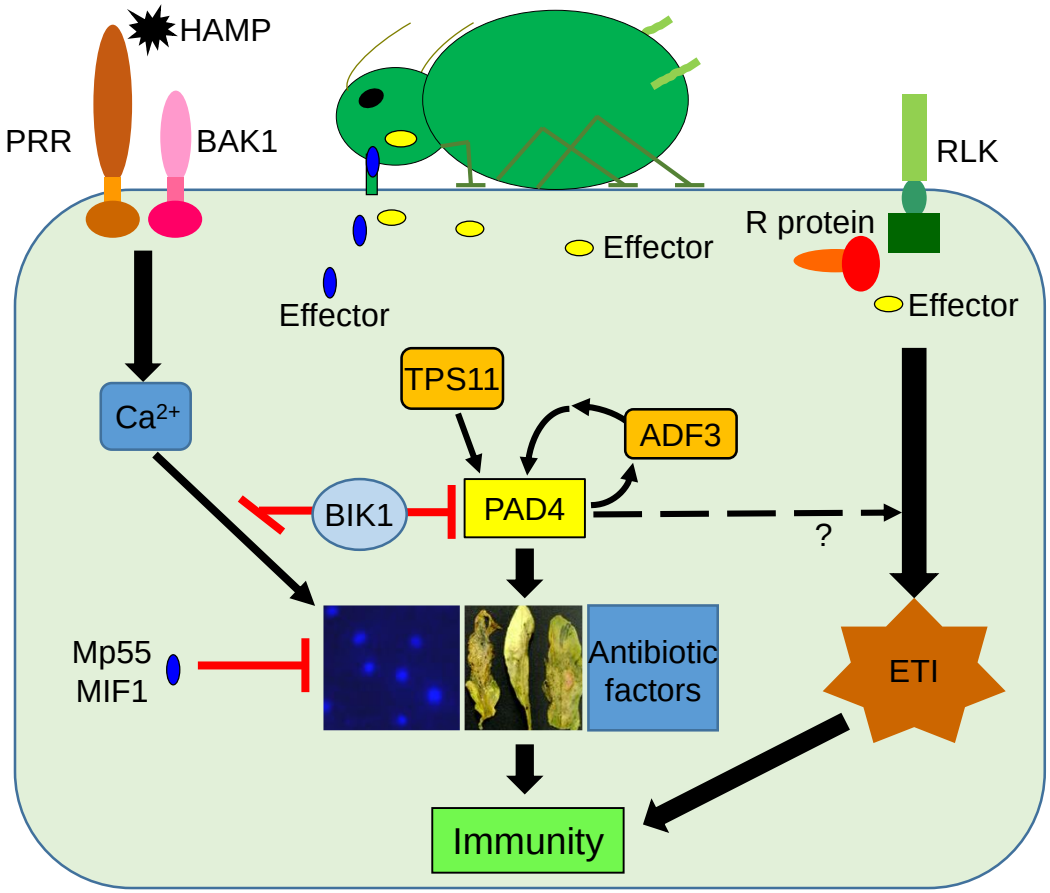

Figure 2. Signaling associated with plant defense against aphid.

This model is based primarily on studies with the Arabidopsis-green peach aphid and the tomato-potato aphid pathosystems. The emerging paradigm for plant immunity to aphids is that plant cells utilize a plasma membrane-localized pattern recognition receptor (PRR) to perceive a conserved herbivore-associated molecular pattern (HAMP) to activate pattern-triggered immunity (PTI). Aphid whole body extracts as well as the microbial GroEL protein can stimulate plant defenses, including defense gene expression and callose deposition. BAK1, which functions as a co-receptor in PTI, is required for the induction of defenses by aphid extracts and GroEL. Aphid whole body extract induced defense involves an influx of Ca^{2+} from apoplastic as well as vacuolar stores. However, how this Ca^{2+} stimulus is relayed leading to the activation of defenses is not known. Aphid-derived effectors, for example, Mp55 and MIF1, are shown to attenuate a subset of defenses associated with PTI. Plants have also evolved Resistance (R) proteins that are involved in the specific recognition of pest-derived effectors that elicit effector-triggered immunity (ETI). In tomato, the R protein Mi1.2, which confers resistance against potato aphid, requires a plasma membrane-localized receptor-like kinase (RLK) SERK1. Arabidopsis PAD4 is a nucleocytoplasmic protein, which is involved in PTI and ETI against pathogens. PAD4 is also essential for basal resistance against the green peach aphid. PAD4 function is required for deterring aphid feeding, callose deposition, the activation of cell death and premature leaf senescence, and accumulation of an antibiotic factor. However, how the PAD4 arm of defense integrates with PTI against aphids is unclear. Moreover, whether PAD4 is required for ETI against aphids is not known. *PAD4* expression, which is upregulated in response to green peach aphid infestation, is regulated by a positive feedback mechanism, which requires ADF3, thus suggesting a role for actin cytoskeleton dynamics in regulation of defense gene expression. *PAD4* expression is also positively regulated by TPS11 and its product trehalose, and is negatively controlled by BIK1, thus indicating that *PAD4* is a node where multiple defense regulatory signals converge.

Table 1. Plant defense against aphids

Biomolecule/structure	Defense activity	Location	References
Surface wax	Physical barrier; defense elicitor	Plant surface	38 - 41
Trichomes	Hinder aphid movement; source of toxic metabolites	Plant surface	43, 44
ACTIN DEPOLYMERIZATION FACTOR 3	Interfere with stylet reaching sieve element	Intercellular space	45
Ascorbate peroxidase	Alter oxidative status of apoplast	Intercellular space	46
Forisome	Sieve element occlusion	Sieve element	50 - 52
SIEVE ELEMENT-LINING CHAPERONE 1	Heat shock protein; Interferes with aphid feeding from sieve elements	Sieve element	56
Callose	Sieve element occlusion	Sieve element	45, 60-68
Lectins	Carbohydrate binding; interfere with gut function	Phloem sap	83-87
Non-protein amino acids (*L*-DOPA, N^6-acetylornithine)	Antibiotic effects	Phloem sap	88, 89
Protease inhibitors	Interfere with gut function	Phloem sap, cell contents	91-94
Cardenolides, cyclic alkaloids	Toxic secondary metabolites;	Phloem sap, cell contents	68, 69
Glucosniolates, benzoxazionids	Toxic secondary metabolites; elicit callose deposition	Cell contents	73-78

Table 2. Aphid saliva-derived elicitors of plant defense

Elicitor	Organism	Host plant	Molecular function	Biological effect	References
Mp10	*Myzus persicae*	*Nicotiana benthamiana*; *N. tabacum*	Unknown	Promote defense signaling	118
Mp42	*Myzus persicae*	*N. benthamiana*; *N. tabacum*	Unknown	Elicit host response; reduce aphid fecundity	118
Mp56	*Myzus persicae*	*Arabidopsis. thaliana*; *Brassica oleracea*; *N. benthamiana*; *N. tabacum*	Predicted retinol dehydrogenase	In planta expression depresses aphid reproduction	61
Mp57	*Myzus persicae*	*A. thaliana*; *B. oleracea*; *N. benthamiana*; *N. tabacum*	Unknown	In planta expression depresses aphid reproduction	61
Mp58	*Myzus persicae*	*A. thaliana*; *B. oleracea*; *N. benthamiana*; *N. tabacum*	Unknown	In planta expression depresses aphid reproduction	61
GroEL	Endosymbiont-*Buchnera aphidicola*	*A. thaliana*; *B. oleracea*; *N. benthamiana*; *N. tabacum; Solanum lycopersicum*	Molecular chaperone (heat shock protein) in bacteria	Promote host defense (callose, reactive oxygen species, PTI marker expression)	60, 61

Table 3. Aphid saliva-derived effectors that facilitate infestation

Effector	Organism	Host plant	Molecular function	Biological effect	References
ApC002	*Acyrthosiphon pisum; Myzus persicae*	*Vicia faba*	Unknown	Essential for aphid feeding from plants	131, 133
Me10	*Macrosihpum euphorbiae*	*Nicotiana benthamiana*; *N. tabacum; Solanum lycopersicum*	Unknown	Promote aphid fecundity	139
Me23	*Macrosihpum euphorbiae*	*N. benthamia-na*; *N. tabacum; S. lycopersicum*	Unknown	Promote aphid fecundity	139
Me47	*Macrosihpum euphorbiae*	*N. benthamia-na*; *N. tabacum; S. lycopersicum*	Glutathione *S*-transferase	Promote aphid fecundity in tomato; stimulate defenses in Arabidopsis	129
MpC002	*Myzus persicae*	*Arabidopsis. thaliana; N. benthamiana*; *N. tabacum*	Unknown	Promote aphid fecundity	118, 134
Mp1	*Myzus persicae*	*A. thaliana*; *Brassica oleracea*; *N. benthamiana*; *N. tabacum*	Binds Arabidopsis VPS52 protein	Promote aphid fecundity	61, 138
Mp2	*Myzus persicae*	*Arabidopsis thaliana*; *Brassica oleracea*; *N. benthamiana*; *N. tabacum*	Unknown	Promote aphid fecundity	61
Mp55	*Myzus persicae*	*Arabidopsis thaliana*; *Brassica oleracea*; *N. benthamiana*; *N. tabacum*	Unknown	Promote aphid reproduction; suppress accumulation of H_2O_2, callose and select glucosinolate in host	61

ACE1	*Acyrthosiphon pisum*	*V. faba*	Homology to angiotensin-converting enzyme	Promote aphid survival, but depress feeding	136
ACE2	*Acyrthosiphon pisum*	*V. faba*	Homology to angiotensin-converting enzyme	Promote aphid survival, but depress feeding	136
Armet	*Acyrthosiphon pisum*	*V. faba*; *N. benthamiana*	Binds Ca^{2+}	Promotes aphid feeding and longevity	135
MIF1	*Acyrthosiphon pisum; Myzus persicae*	*V. faba*; *N. benthamiana*	Cytokine like; homologous to macrophage migration-inhibition factor	Suppress host defense; promote aphid feeding and fitness	62